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EFFECT OF BLACK CUMIN OIL ADMINISTRATION ON CORTISOL LEVEL AND LIVER HISTOPATHOLOGY OF HEAT STRESSED BROILER CHICKENS

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ABSTRACT

The aim of this study was to observe cortisol levels and liver histopathology of broiler chicken that were treated with black cumin oil (BCO) under heat stress. A total of 15 broiler chickens were used in this study and divided into 5 groups, K- (without treatment), K+ (given heat stress), P1 (given heat stress and 0.56mL BCO/400kg body weight, P2 (given heat stress and 1.11 mL BCO/400 g body weight), and P3 (given heat stress and 2.22 mL BCO/400 g body weight). Heat stress was given for 5 hours with temperature range of 34-35° C for 7 days. Cortisol was measured using the cortisol enzyme-linked immunosorbent assay (ELISA) kit. Liver histopathology was stained with hematoxylin eosin and observed with electron microscope. The data were analyzed using one way analysis of variance (ANOVA). This study found that application of heat stress to broiler chickens increased cortisol levels and induced histopathological changes in the liver. The BCO administration reduced cortisol level significantly ($P<0.05$) in heat-stressed broilers. BCO administration also significantly reduced ($P<0.05$) the degenerative changes in liver histopathology such as fat degeneration, hemorrhage and necrosis in broiler chickens under heat stress, but did not significantly influence the inflammatory cells infiltration. As conclusion, BCO administration to broiler chickens under heat stress can reduce cortisol levels and minimize histopathological changes in the liver.

Key words: black cumin, broiler chicken, cortisol, histopathology

ABSTRAK

Tujuan penelitian ini untuk mengamati kadar kortisol dan gambaran histopatologi organ hati ayam broiler yang diberi minyak jintan hitam (MJH) dalam kondisi stres panas. Sebanyak 15 ekor ayam broiler dalam penelitian ini dibagi menjadi 5 kelompok, Kelompok K- (tanpa perlakuan), kelompok K+ (diberi stres panas), kelompok P1 (stres panas dan MJH 0,56ml/400 kg bobot badan, kelompok P2 (stres panas dan MJH 1,11 ml/400 g bobot badan), dan kelompok P3 (stres panas dan MJH 2,22 ml/400 g bobot badan). Stres panas diberikan selama 5 jam dengan kisaran suhu 34-35 °C selama 7 hari. Kortisol diukur menggunakan kit kortisol metode enzyme-linked immunosorbent assay (ELISA) dan pembuatan histopatologi organ hati dengan pewarnaan haematoxylin eosin dan diamati dengan menggunakan mikroskop elektron. Kadar kortisol dianalisis dengan menggunakan analisis varians pola satu arah. Hasil penelitian menunjukkan bahwa pemberian stress panas pada ayam broiler dapat meningkatkan kadar kortisol dan perubahan histopatologi pada organ hati. Pemberian MJH berpengaruh nyata ($P<0,05$) dalam menurunkan kadar kortisol pada ayam broiler yang diberi stres panas. Pemberian MJH juga menunjukkan pengaruh nyata ($P<0,05$) dalam meminimalisir perubahan sel hati yang berupa gambaran degenerasi lemak, hemoragi dan nekrosis pada ayam broiler yang diberi stres panas, tetapi tidak berpengaruh nyata dalam meminimalisir infiltrasi sel radang. Hasil penelitian dapat disimpulkan bahwa. pemberian MJH pada ayam brolier yang mengalami stres panas dapat menurunkan kadar kortisol dan meminimalisir perubahan histopatologi pada organ hati.

Kata kunci: jintan hitam, ayam broiler, kortisol, histopatologi

INTRODUCTION

One of the highest growing sectors in livestock industry is broiler farming. However, broiler farming production could be inhibited by high environmental temperature (Andriyana, 2011). Physiological changes to heat stress such as increased cortisol level can be used as an indicator for heat stress in broiler chickens (Dehnhard *et al*., 2003; Swathi *et al*., 2012). According to Sugito *et al*. (2007), administration of heat stress at a temperature of 33±1° C for 2 and 4 hours can increase cortisol secretion in the feces. Heat stress can also cause oxidative reactions due to free radicals formation which can cause cellular damage (Kregel and Zhang, 2007; Maini *et al*., 2007). A study on heat stress by Sugito *et al*. (2007) found several histopathological changes in the liver of broiler chickens such as fat degeneration, necrosis and inflammatory cells infiltration.

Black cumin (*Nigela sativa*) is an herbal plant that is rich in antioxidants, one of which is thymoquinone, which can remove free radicals (Kruk *et al*., 2000). Some researchers have proven the efficacy of black cumin as an antidepressant in mice (Perveen *et al*., 2014). The antioxidants contained in black cumin oil can provide hepatotoxic and nephrotoxic effects in vivo and in vitro. Black cumin oil significantly improves histological parameter and function in reducing oxidative stress induced by cyclosporine A. Black cumin oil protects kidney tissues from free radicals and prevents kidney dysfunction (Uz *et al*., 2008). Based on the description above, this study was conducted to investigate the cortisol levels and liver histopathology of broiler chicken that were treated with black cumin oil (BCO) under heat stress.

MATERIALS AND METHODS

A total of 15 (fifteen) 2-week-old broiler chickens, with an average weight of 350-400 g, were used as samples in this study. The chickens were then divided

into 5 groups, namely K- group (without treatment), K+ group (given heat stress), P1 group (given heat stress and 0.56 mL BCO/400kg body weight, P2 group (given heat stress and 1.11 mL BCO/400 g body weight), and P3 group (given heat stress and 2.22 mL BCO/400 g body weight). Heat stress was given for 5 hours with temperature range of 34-35° C for 7 days. Serum samples were taken on the 8th day for examination of cortisol level using the cortisol enzyme-linked immunosorbent assay (ELISA) kit in accordance to the protocol. The entire sampling process was carried out at the Research Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala in Banda Aceh. Liver sampling was carried out on the 8th day after the chicken was necropsied and then fixated in 10% formalin solution. Histopathological preparations were made in accordance to the standard protocol of Pathology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh and stained with hematoxylin eosin before observed using an electron microscope.

Data Analysis

The data were analyzed with one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Cortisol Levels

The effect of BCO on cortisol levels can be seen in Tabel 1. It can be seen from Tabel 1 that the highest cortisol level was in the K+ group (5.92 µg/ml), and the level subsequently decreases in groups that treated with black cumin for 7 days (P1= 4.20, P2= 3.28, and P3= 3.11 µg/ml), while the lowest value was found in the K- group (2.31 µg/ml). Average cortisol levels between K- and K+ groups showed a significant difference. There were no significant differences among P1, P2, and P3 groups, however P2 and P3 showed significant differences with K+ group.

Under stress conditions, cortisol level increases accompanied by an increase in body temperature (Schaltter *et al*., 2002). Stress can increase ACTH hormone (adrenocorticotropine) level, resulting in a 20-fold increase in cortisol secretion (Astutik and Elfi, 2014). Cortisol is also closely related to serotonin levels in the brain (Figuerido, 2003). Cortisol levels in this study were measured through blood serum. Higher level of cortisol can be interpreted as a state of depression in test animals (Gerra *et al*., 2001). The antidepressant activity of BCO could be seen by the decrease in cortisol level in heat-stressed test animals.

The lower cortisol levels in treatment group compared to the control group showed the influence of oral BCO administration. This is in accordance with the report of Perveen *et al*. (2014), which stated that one of the compounds contained in black cumin, the coumarin, has pharmacological effects as an antidepressant. These compounds increase the synthesis of 5-HT (5-hydroxytrytamine or serotonin) and at the synapses play a role in increasing plasma tryphophan concentration in the brain. A study by Randhawa and Alenazi (2016) also reported an antidepressants effect of BCO on mice tested in a labyrinth of cages. The result of the study showed an increase in 5-HT levels in the brain, tryptophan brain levels also increased significantly. Other studies also reported that black cumin acts as an antidepressant through increased serotonin (5-HT) (Ahmad *et al*., 2013). Other substances contained in BCO are alkaloids and flavonoids (Mangesha, 2015). Alkaloid compounds show antidepressant activity which plays a role in increasing serotonin levels by reducing adrenocorticotropic hormone level (Lee *et al*., 2005; Fortunato *et al*., 2010; Mao *et al*., 2011).

Based on the analysis result of P1 treatment group that was given BCO at a dose of 0.56 mL/400 g body weight, administration of BCO showed a significant reduction in cortisol level. Decreased cortisol levels can also be caused by other active compounds in black cumin, such as alkaloids and flavonoids. According to Yi *et al*. (2010), flavonoids generally have antidepressants effect by increasing serotonin (5-HT) and norepinephrin (NE) in the brain (Tian *et al*., 2010; Machado *et al*., 2012). According to Syarif *et al*. (2011), increased brain serotonin and norepinephrin

Tabel 1. Average (±SD) serum cortisol level based on treatment groups

Treatment group	Cortisol (µg/mL)
K-	2.31 ± 0.03^{a}
K+	5.92 ± 0.74^{b}
P1	4.20 ± 1.06^{ab}
P2	3.28 ± 1.81^{a}
P3	3.11 ± 0.50^{a}

$^{a, b, ab}$Different superscripts within the same column indicate significant difference (P<0.05)

Table 2. The average value (±SD) of histopathological changes in the liver

Treatment group	Fat degeneration	Hemorrhage	Necrosis	Inflammatory cell infiltration
	Average (±SD)	Average (±SD)	Average (±SD)	Average (±SD)
K-	0.21 ± 0.03^{a}	0.53 ± 0.02^{b}	0.46 ± 0.15^{b}	4.47 ± 0.26^{a}
K+	0.33 ± 0.06^{b}	0.67 ± 0.10^{c}	0.54 ± 0.07^{b}	4.49 ± 0.26^{a}
P1	0.21 ± 0.81^{a}	0.41 ± 0.02^{a}	0.35 ± 0.47^{a}	4.46 ± 0.34^{a}
P2	0.33 ± 0.29^{b}	0.51 ± 0.03^{ab}	0.51 ± 0.19^{b}	4.46 ± 0.15^{a}
P3	0.29 ± 0.51^{ab}	0.46 ± 0.00^{b}	0.52 ± 0.10^{b}	4.47 ± 0.18^{a}

$^{a, b, ab}$Different superscripts within the same column indicate significant difference (P<0.05)

levels will improve mood, increase physical activity and increase appetite. Sugito (2007) also stated that the flavonoids can reduce heat stress in chickens through vasodilation to improve evaporation.

Liver Histopathology

Histopathological examinations on chicken liver found several microscopic features such as fat degeneration, hemorrhage, necrosis and inflammatory cells infiltration. The average value of liver histopathology changes from statistical analysis result can be seen in Table 2. Based on Table 2, BCO administration to broiler chickens under heat stress showed significant differences ($P<0.05$) in average values of fat degeneration, hemorrhage and necrosis in the liver, however there was no significant difference ($P>0.05$) on the average value of inflammatory cell infiltration. In this study, the highest rate of change in liver histopathology is fat degeneration which was 0.33 in K+ and P2 groups, 0.29 in P3 group and was the lowest in K- and P1 groups, 0.21. Whereas for hemorrhage, the highest level was in the K+ group, 0.67, followed by 0.53 in K- group, 0.51 in P2 group, 0.46 in P3 group and the lowest in P1 group, 0.41. The highest level of necrosis was found in the K+ group, 0.54, followed by 0.52 in P3 group, 0.51 in P2 group, 0.46 in K-group and the lowest in P1 group, 0.35. The highest inflammatory cell infiltration was found in the K+ group (4.49), followed by K- and P3 groups (4.47), and the lowest in P1 and P2 treatment groups (4.46).

There was a significant difference in average value of fat degeneration between K- and K+ groups. P1 and P2 treatments had no significant difference to P3, but P1 was significantly different from K+ group. The

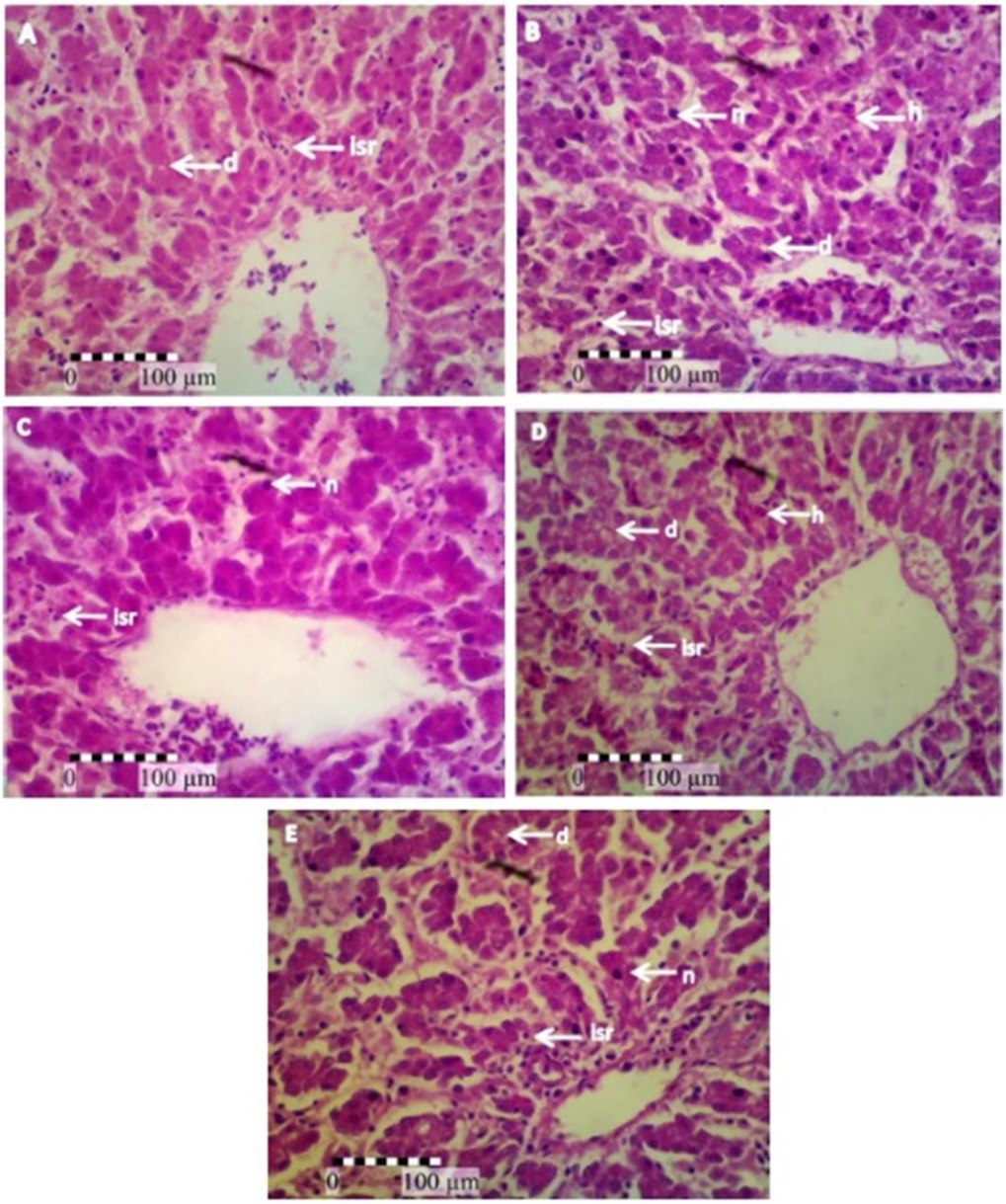

Figure 1. Histopathological image of chicken liver. A= K- group without treatment, B= K+ group that was given heat stress; C = P1 group that was given heat stress and 0.55 mL black cumin/400 kg body weight, D = P2 group that was given heat stress and 1.11 mL black cumin/400 kg body weight, E= P3 group that was given heat stress and 2.22 mL black cumin/400 kg body weigh d= Fat degeneration, n= Necrosis, isr= Inflammation cell infiltration, h= Hemorrhage. HE, 400x

statistical analysis of average hemorrhage values showed a significant difference between the K- and K+ groups. The P1 group was significantly different from P2 and P3 group, and also from the K- and K+ groups. Based on statistical analysis result of the average value of liver cell necrosis, there was no significant difference between the K- and K+ groups. The P1 treatment was significantly different from P2 and P3, and also significantly different from the K- and K+ groups. The results of statistical test on the average value of inflammatory cell infiltration did not show a significant difference between each groups.

Based on the results of histopathological observations of chicken liver under heat stress (Figure 1), histopathology in the control group K- showed fat degeneration such as vacuoles and inflammatory cell infiltration (A), while fat degeneration, necrosis, hemorrhage and inflammatory cell infiltration were found in the K+ group that was given heat stress found (B). Histopathological feature of the P1 group that was given 0.55 mL of BCO and heat stress showed normal hepatocyte cells with few necrotic cells and few inflammatory cell infiltrations (C). In P2 group that was given 1.11 mL of BCO and heat stress, there were fat degeneration, hemorrhage, and inflammatory cell infiltration (D). Histopathological feature of the P3 group that was given 2.22 mL of BCO and heat stress were fat degeneration, necrosis, and inflammatory cell infiltration around blood vessels were seen (E).

Liver is the main organ involved in metabolic processes. One of the metabolic disorders that can cause hepatocyte cell disorders is reactive oxygen species (ROS) formation (Fernandez-Checa and Kaplowitz, 2005). Oxidative stress can occur due to high environmental temperature, giving rise to excessive free radicals (Miller *et al*., 1993). Free radicals or ROS can be formed through enzymatic or metabolic pathways. The process of change from arachidonic acid to prostaglandin and prostacyclin is triggered by lipoxygenase and cyclooxygenase enzymes which produce reactive oxygen compounds in the form of peroxides and epoxides, and oxidase in the form of aldehyde oxidase and subsequently form superoxide anion radicals (Sayuti and Rina, 2015).

Previous study by Aengwanich and Simaraks (2004) has shown liver cell damage due to high environmental temperatures in the form of fat degeneration, necrosis and infiltration of granulated leukocytes in the liver tissue. Fat degeneration is a morphology change that cause reduction in liver function and is caused by accumulation of fat in the liver cells cytoplasm until small patches of clear fat can be seen (Dannuri, 2009). If fat degeneration continues, the cell can experience necrosis (Wulandari *et al*., 2007). Cells that experience necrosis will release various mediators that will initiate inflammatory process and attract inflammatory cells (Kumar *et al*., 2009). Hemorrhage is an advanced stage of congestion that occurs because sinusoids lost the ability to contain blood, resulting in sinusoid stretch which can eventually rupture (Sudiono *et al*., 2003).

Based on the results of statistical analysis, it can be concluded that BCO administration can minimize damage by heat stress to liver cells. Thymoquinone, as the main active substance in black cumin, is responsible for the hepatoprotective effect through antioxidant and anti-inflammatory properties. Several studies have shown the protective effects of thymoquinone against liver cell damage caused by ROS by increasing antioxidant defenses in the body which acts to clear free radicals in the body (Mollazadeh and Hosseinzadeh, 2014).The antioxidant mechanism of thymoquinone is through reduction of nitric oxide and inducing the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST) and depletes reduced glutathione (GSH) (Mabarouk and Cheikh, 2016).

The average results of each histopathological change showed that P1 group had a lower average compared to P2 and P3. Based on this statement, it meant that the administration of 0.55 mL BCO was worse compared to doses of 1.11 mL and 2.22 mL in minimizing hepatocellular damage. This could be explained by the excessive amount of antioxidant at the higher dose, resulting in an imbalance between oxidants and antioxidants. This imbalance can cause a disturbance in the physiological concentration of ROS in the cells that is for normal cellular functions. This excessive antioxidant will disrupt cellular physiology and ultimately be toxic to organs (Bouayed and Bohn, 2010).

A study by Yildiz *et al*. (2008) study also found that administration of black cumin was capable to suppress pathological changes and hepatocellular damage due to injury. Antioxidant such as thymoquinone works as a purifier of reactive oxygen species such as superoxide radicals and hydroxyl radicals (Badary *et al*., 2002). Burits and Bucar (2000) tested essential oils from black cumin using two-dimensional thin layer chromatography method and found that the thymoquinone in black cumin had the ability to scavenge free radicals that is effective in non-enzymatic lipid peroxidation and deoxyribose degradation. Badary *et al*. (2002) also proved that thymoquinone was able to inhibit microsomal lipid peroxidation and these substances were more actively act as superoxide anion scavenger.

In Susianti (2013) study, administration of black cumin extract was able to inhibit free radicals from damaging the alveolar cell wall, minimizing cell damage. According to El-Tahir and Bakeet (2006), black cumin extract can prevent alveolar damage from antioxidants and had anti-inflammation effect. Thymoquinone and nigelon in cumin oil can reduce inflammatory reactions through antioxidant activity (El-Dakhakhny *et al*., 2002). The anti-inflammatory mechanism in black cumin is inhibition of cyclooxygenase II and 5-lipoxygenase in the arachidonic acid metabolic pathway and lipid-bearing peroxidation (Mohammad *et al*., 2011). Thymoquinone

also has anti-apoptotic effects by reducing inflammatory mediators, hence it can prevent cellular damage. The repair mechanism involves a decrease in the inflammatory response by decreasing levels of tumor necrosis factor alpha (TNF-α), nuclear factor kappa B (NF-kB) and cyclooxygenase 2 (COX-2) (El-Sheikh *et al*., 2015).

CONCLUSION

Administration of BCO to broiler chickens that experienced heat stress reduces cortisol levels and minimizes histopathological changes in the liver.

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